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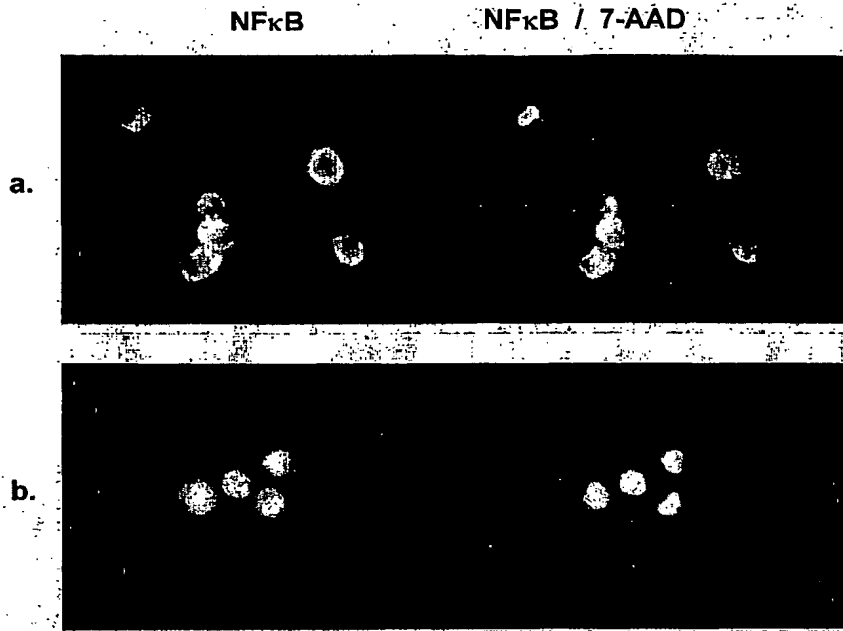


Figure 1: Visualization of NF κ B Nuclear Translocation in A549 Cells Using Immunofluorescence Microscopy

TNF- α and IL-1 β stimulation initiates a signaling cascade that results in the translocation of NF κ B from the cytoplasm to the nucleus of the adherent human carcinoma cell line A549 cells. Untreated A549 cells (a) and A549 cells treated with TNF- α (2 ng/ml) and IL-1 β (10 pg/ml) for 1 hour (b) were trypsinized and probed for NF κ B expression and nuclear morphology. Briefly, the cells were fixed in 4% paraformaldehyde, permeabilized with 0.1% triton, and incubated with mouse anti-NF κ B (p65) + Alexa Fluor[®] 488 donkey anti-mouse IgG. Cells were washed and resuspended in 1% paraformaldehyde containing 7-AAD, then mixed with an equal volume of antifade and visualized on slides using a Nikon Eclipse E600 fluorescence microscope equipped with bandpass filters appropriate for FITC (535/40 nm) and 7-AAD (630/60 nm) fluorescence. NF κ B images in grey are depicted on the left. NF κ B (green) / 7-AAD (red) composite images on the right demonstrate the nuclear localization of NF κ B following TNF- α / IL-1 β treatment.

Fig. 1

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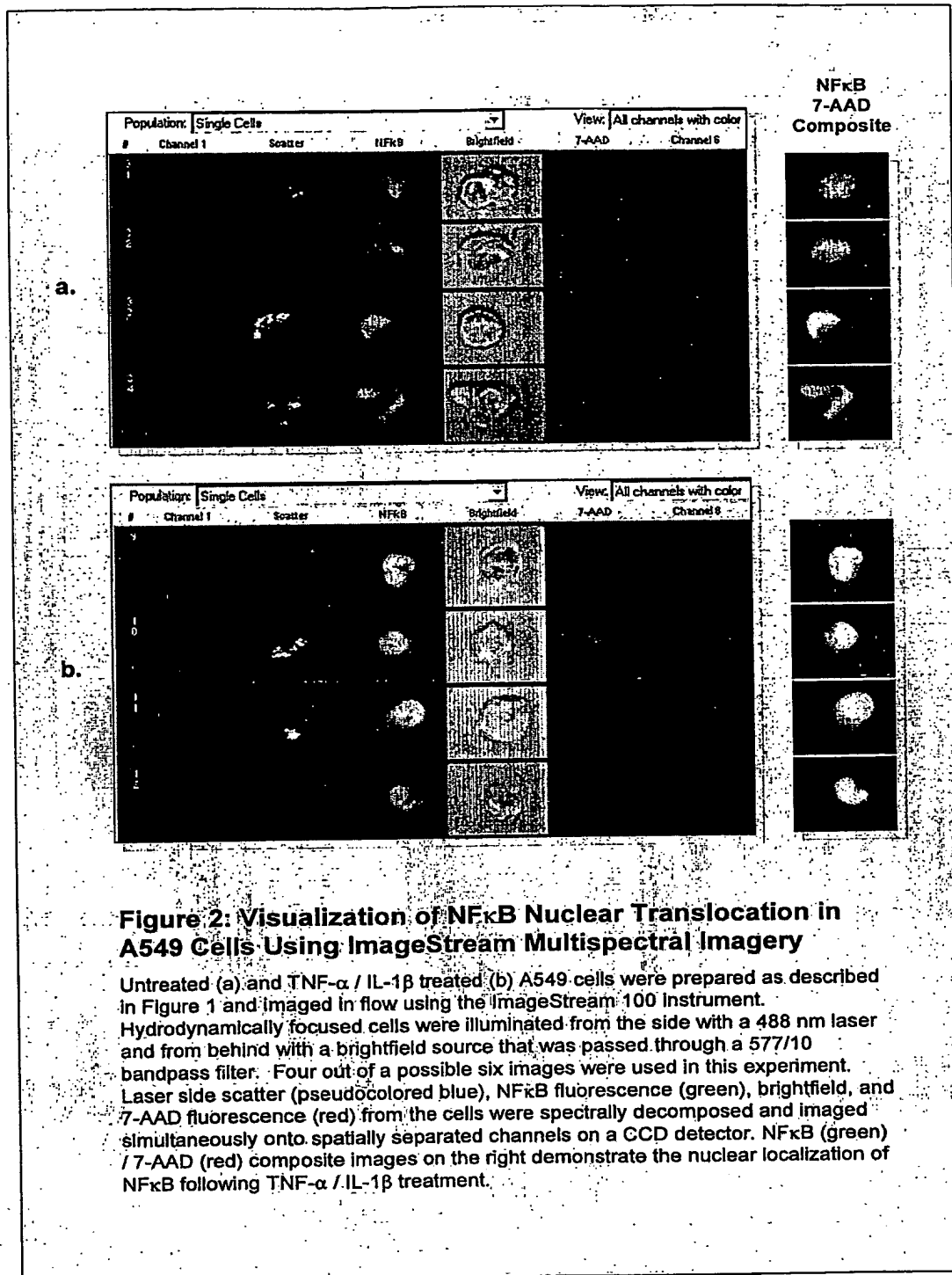


Fig. 2

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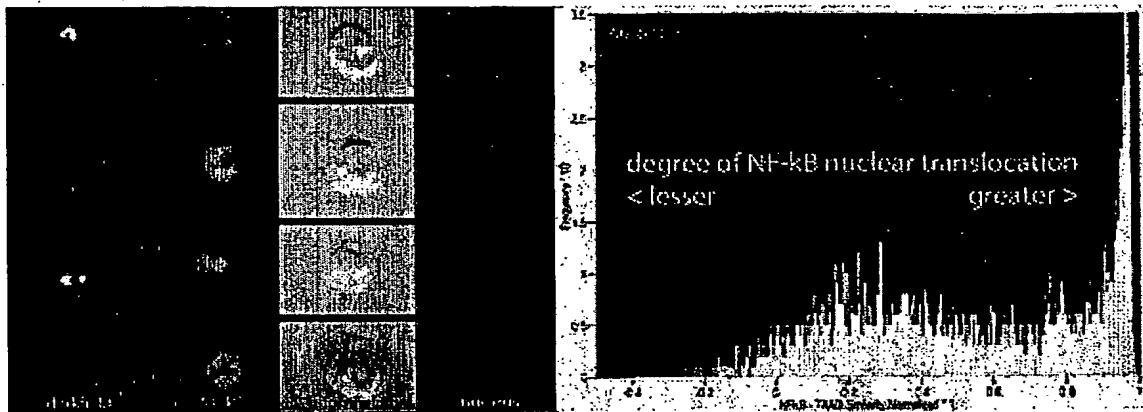


Figure 2C. NF-kB Nuclear Translocation in Immune Cells

The data (above left) show cells imaged simultaneously in darkfield, green fluorescence, brightfield, and red fluorescence. The sample consisted of a monocytic cell line stained with an antibody against the NF- κ B transcription factor (green) as well as a nuclear stain (red). Cells treated with lipo-polysaccharide (image rows 2-4) exhibit translocation of NF- κ B from the cytoplasm to the nucleus while untreated cells lack NF- κ B in the nuclear compartment (top row). A statistical analysis of imagery from 6616 cells quantitatively characterizes the degree of NF- κ B nuclear translocation in the sample. Amnis' ImageStream platform is the only cell analysis technology that can perform this valuable assay on immune cells in suspension.

Fig. 2C

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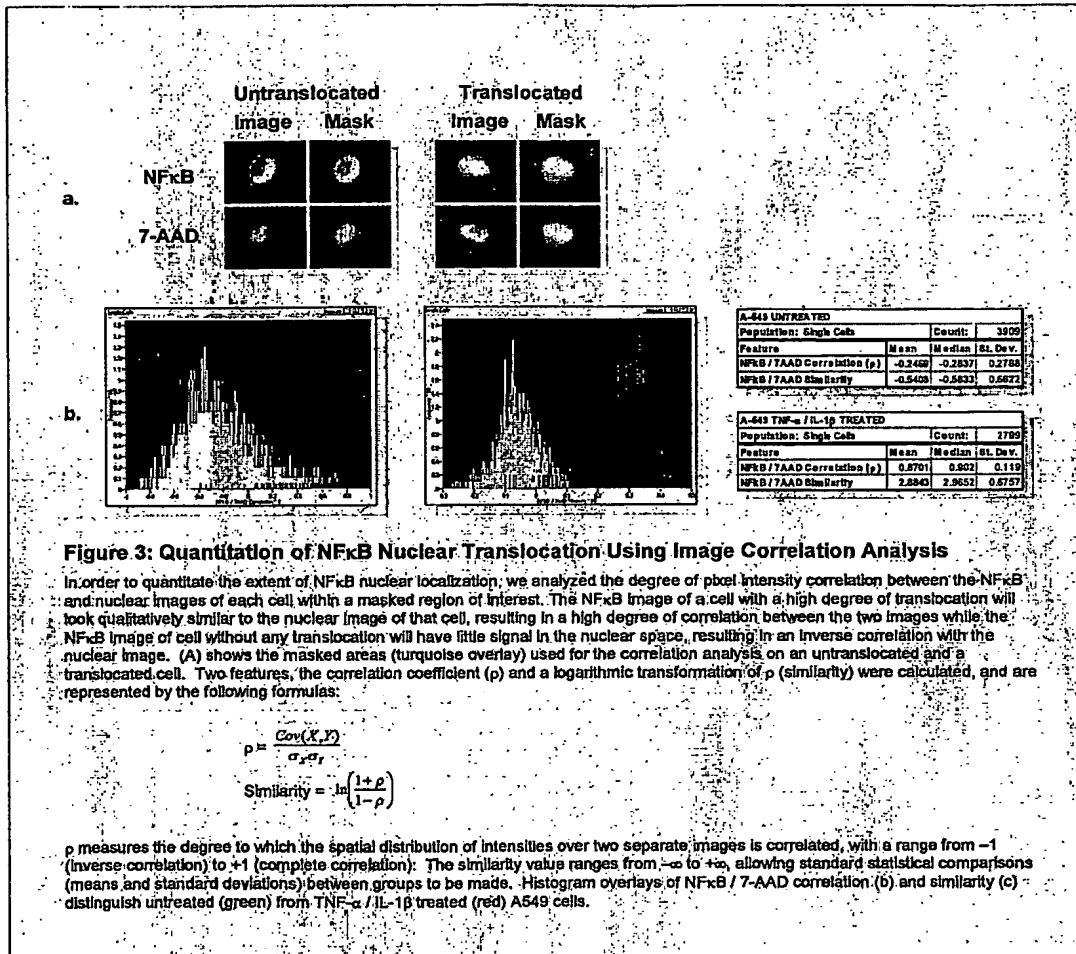
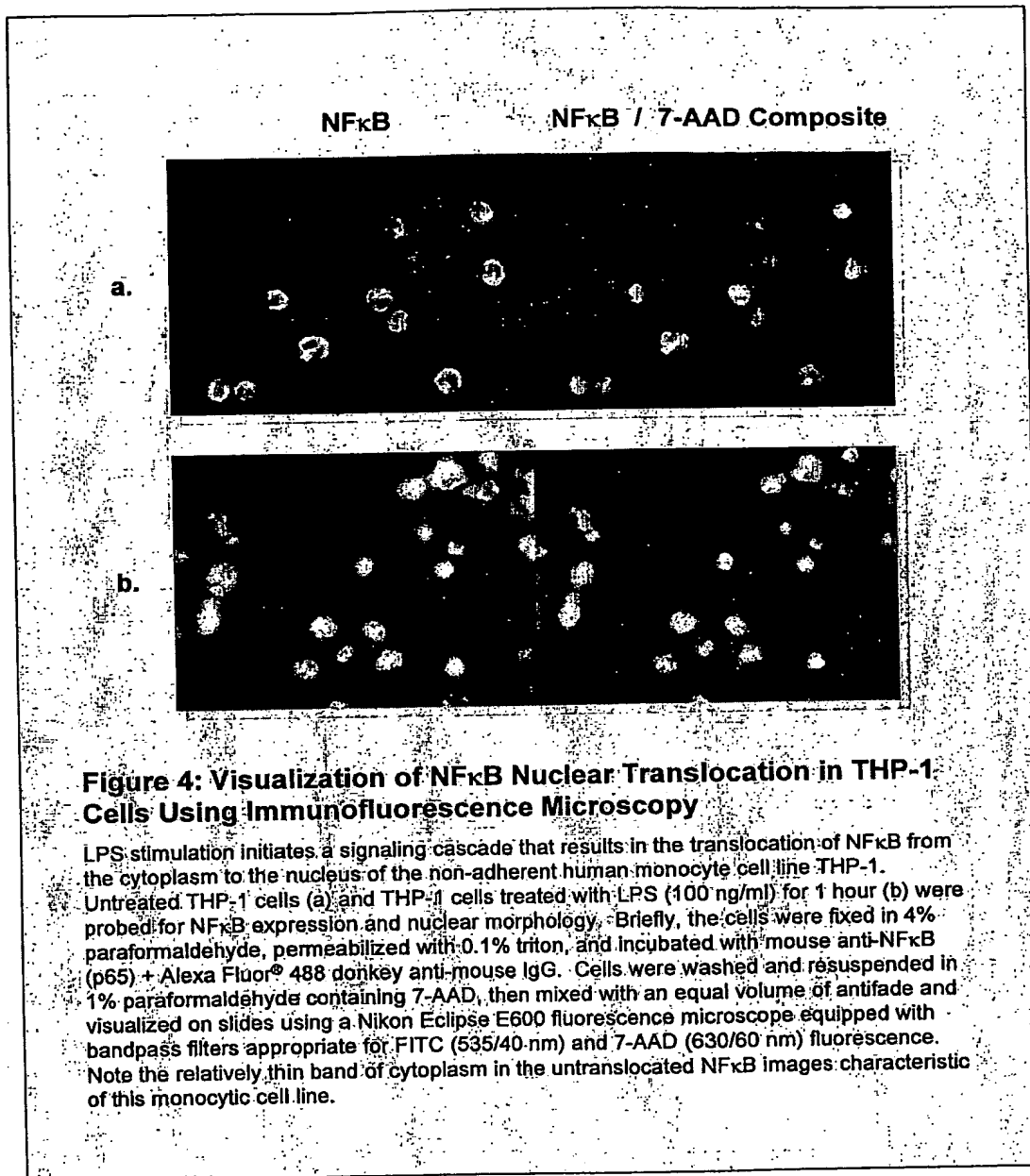


Fig. 3

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**Fig. 4**

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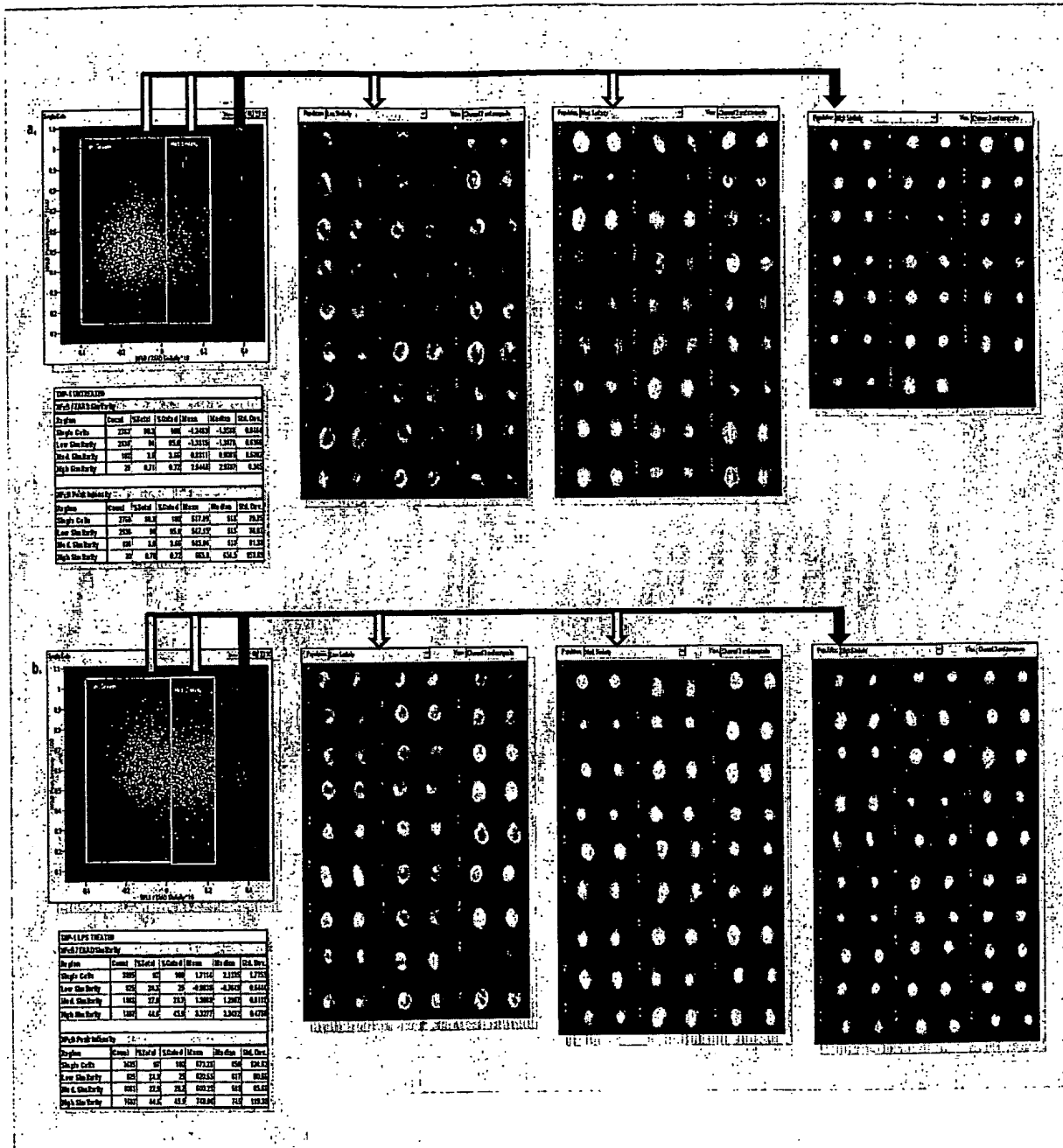


Fig. 5

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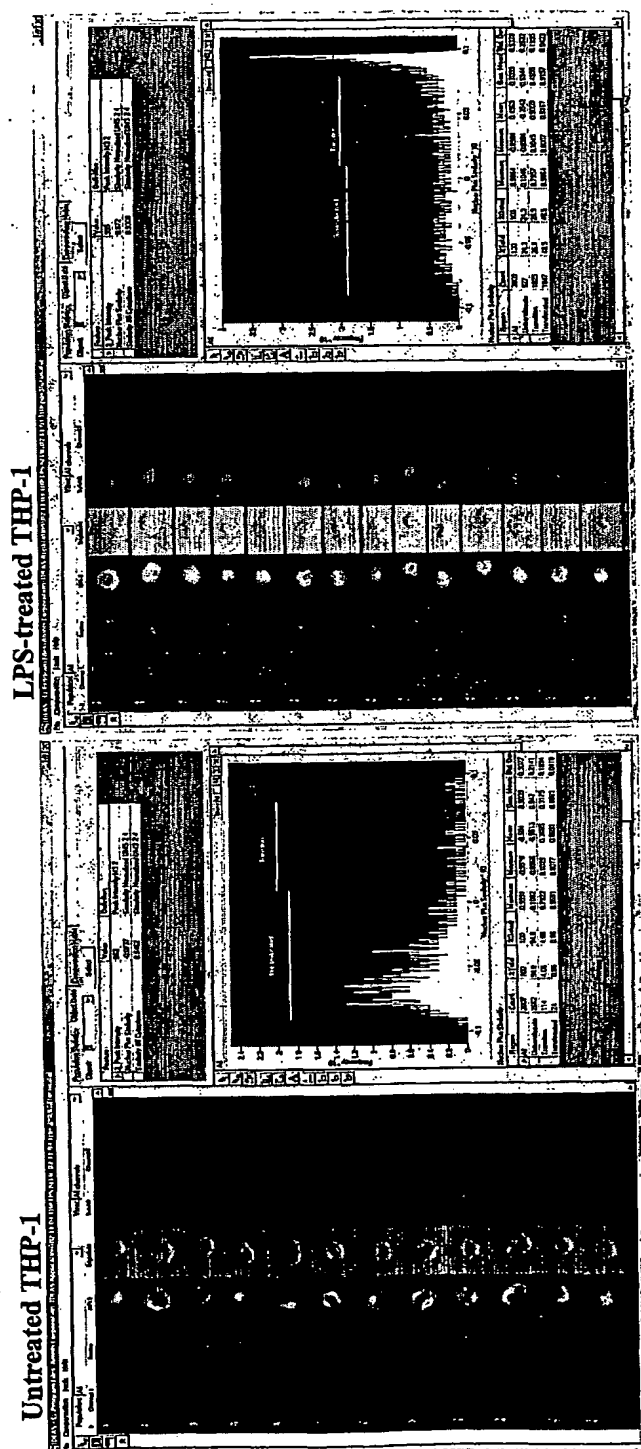
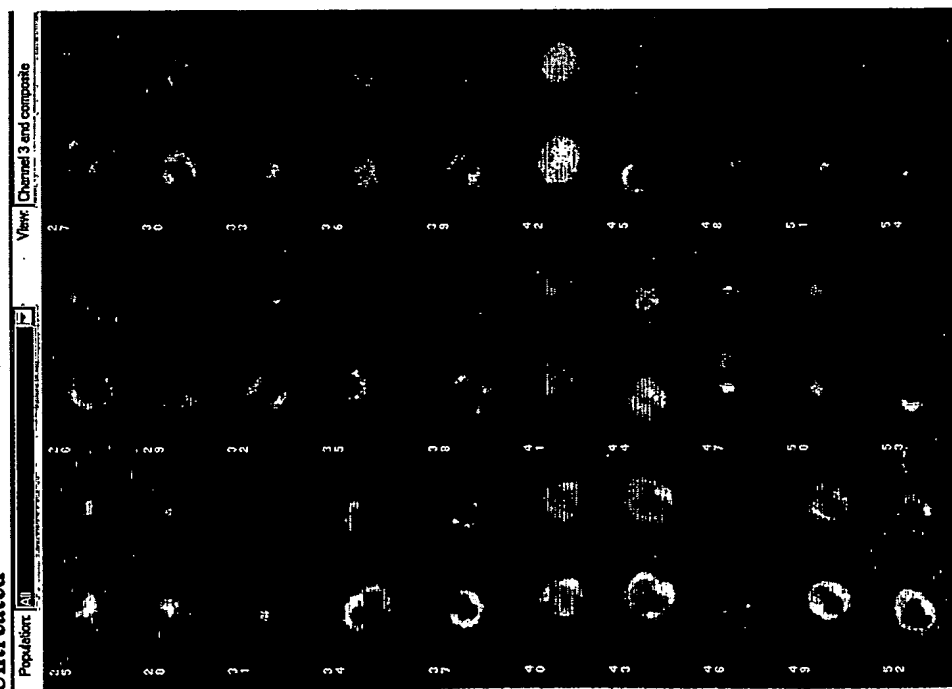


Fig. 6

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THP-1 NFkB/7AAD Composite

Untreated



LPS-Treated

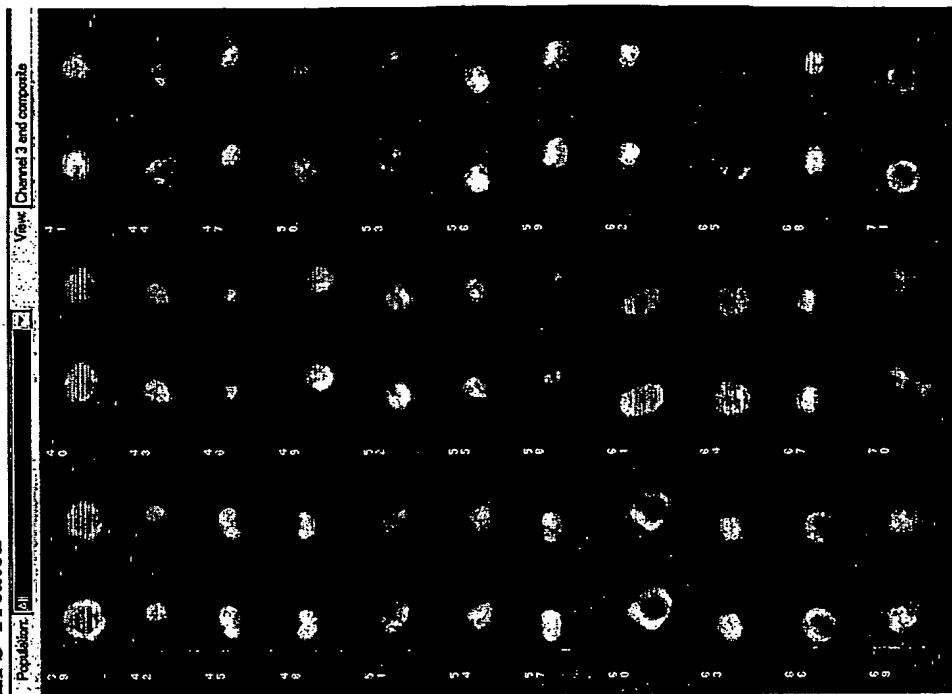
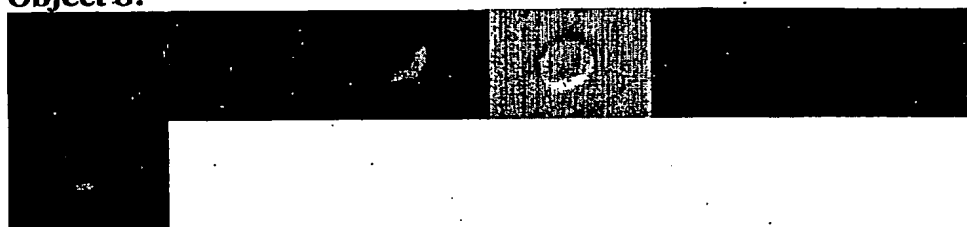


Fig. 7

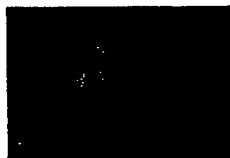
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Object 8:**7-AAD stain**

MASKS:



85% UM3



75% UM5



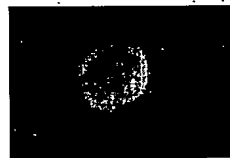
Standard M5

NFκB stain

MASKS:



85% UM3

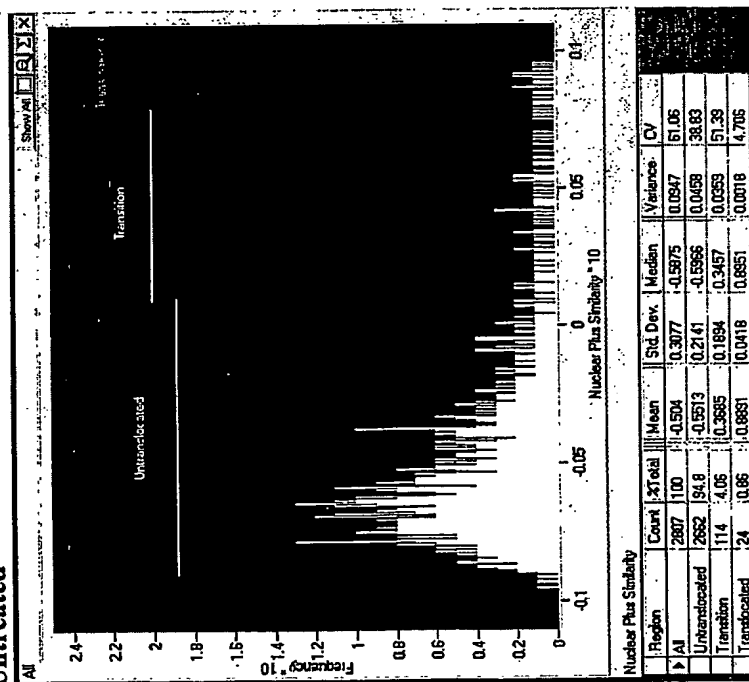


75% UM5

Feature	Value	Definition
▶ 3_Peak Intensity	571	Peak Intensity M3 2
Nuclear Plus Similarity	-0.155	Similarity Normalized UM5 2 4
Similarity 85 Cytoplasm	0.5003	Similarity Normalized UM3 2 4

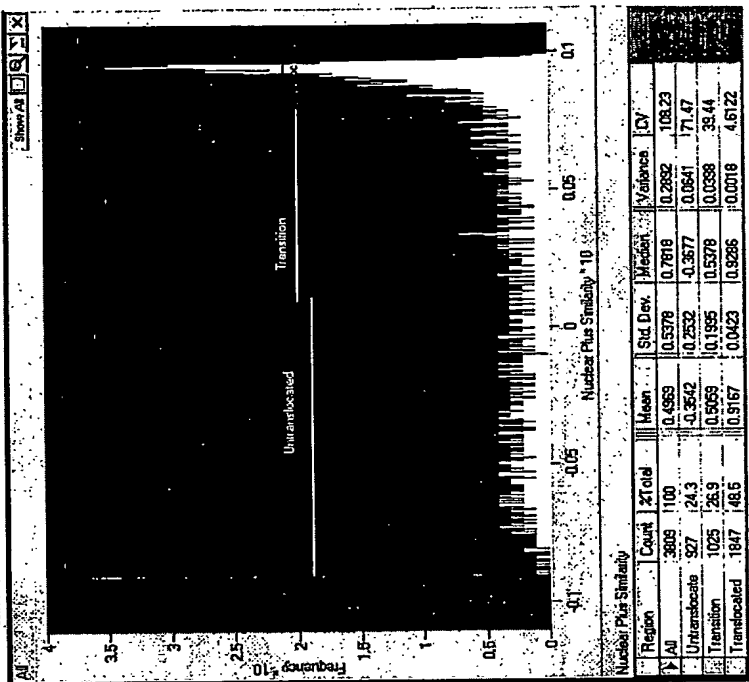
Fig. 8

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COMPARTMENTAL CORRELATION FEATURE:UntreatedMedian Compartmental Correlation Feature:

Untranslocated = -0.5966 +/- 0.2141

Difference of 1.5252

LPS-treated

Translocated = 0.9286 +/- 0.0423

Fig. 9

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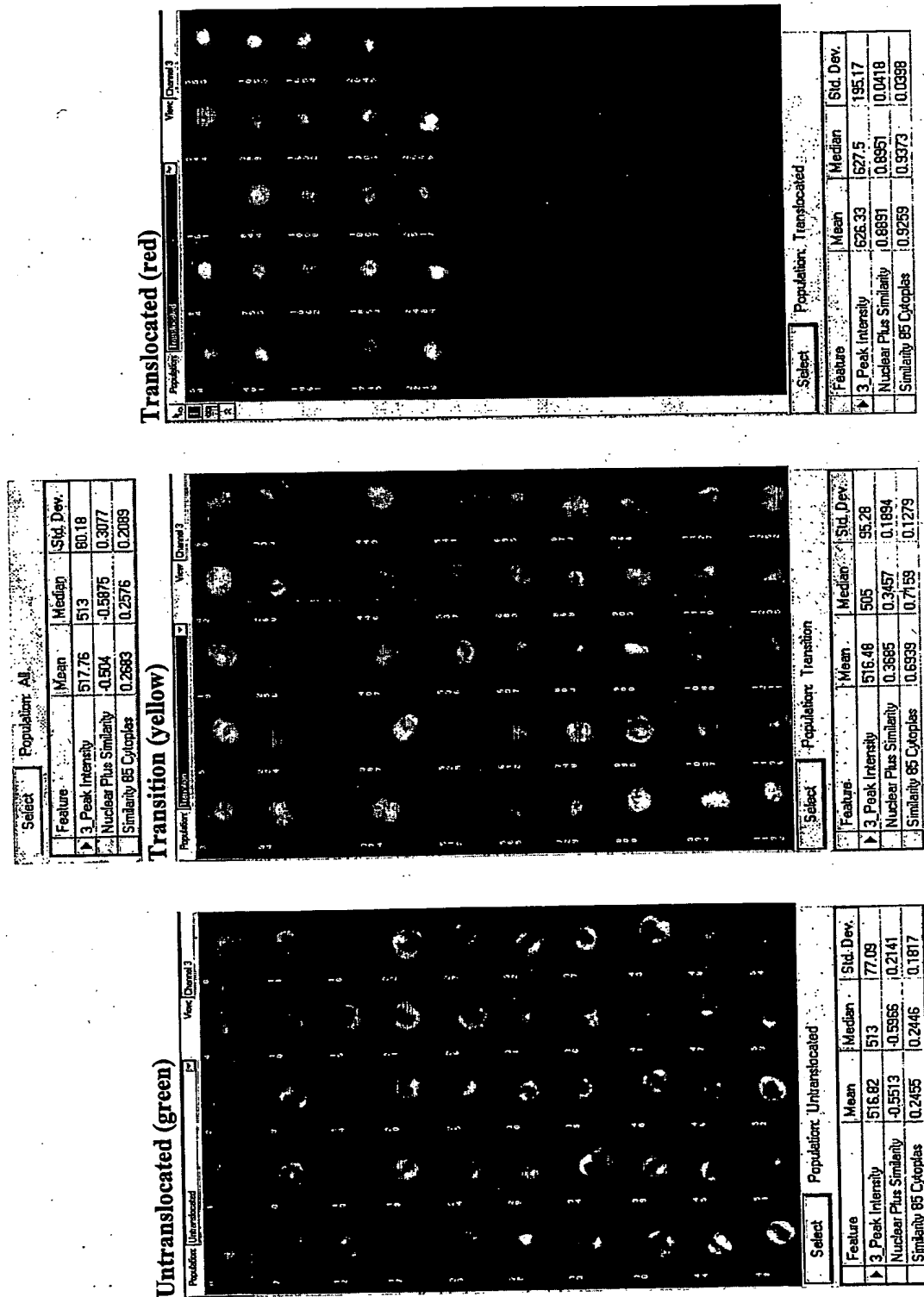


Fig. 10A

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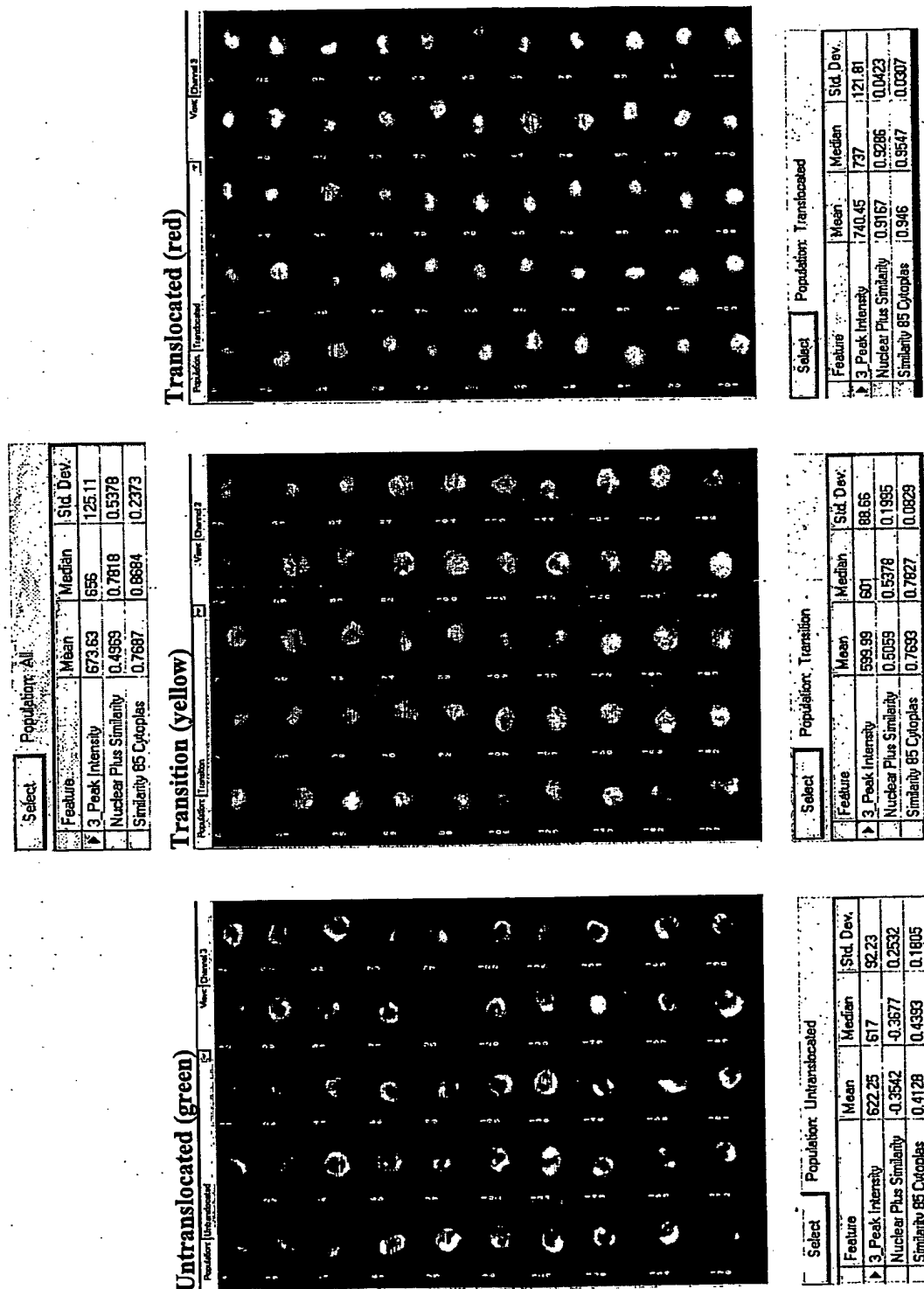


Fig. 10B

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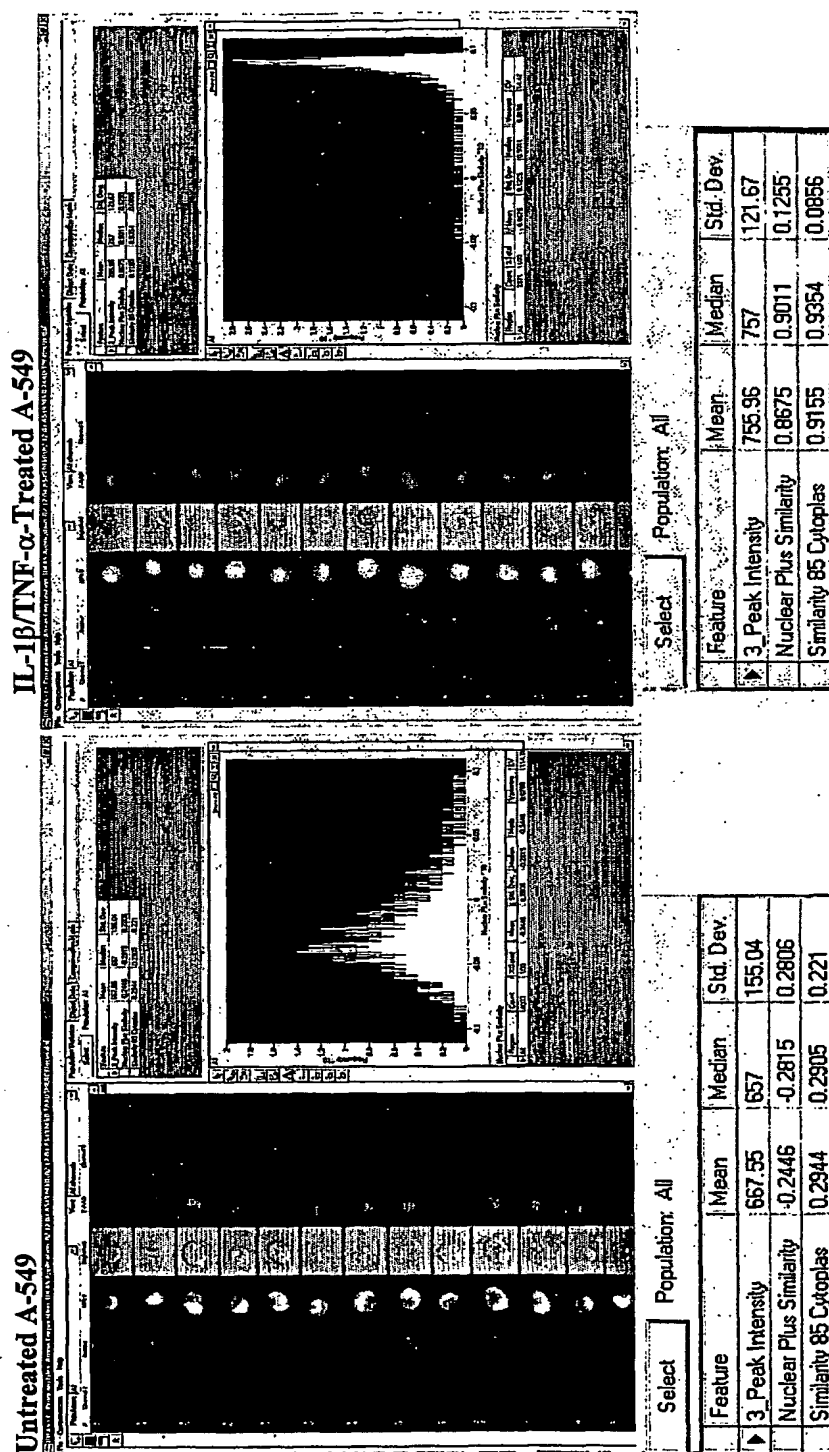


Fig. 11

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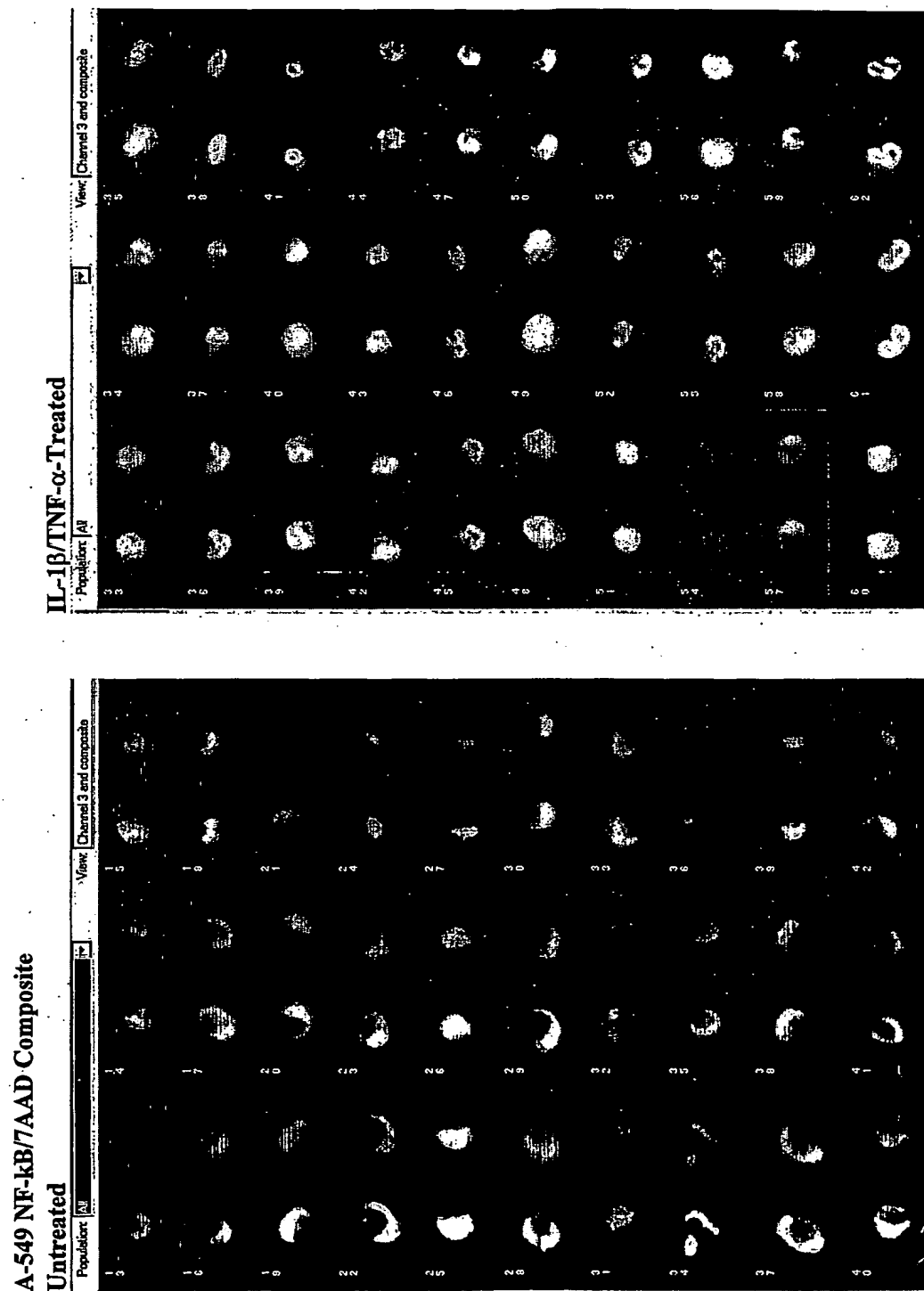
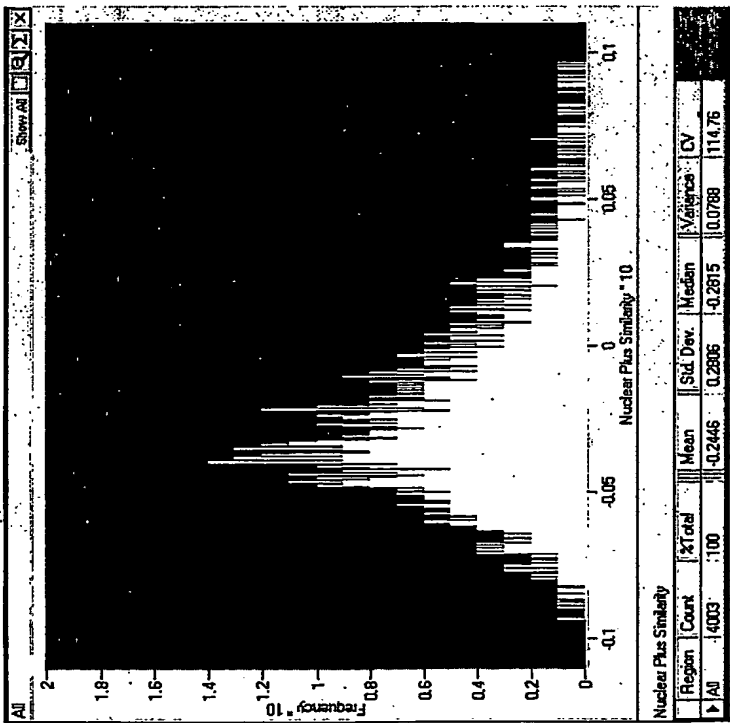


Fig. 12

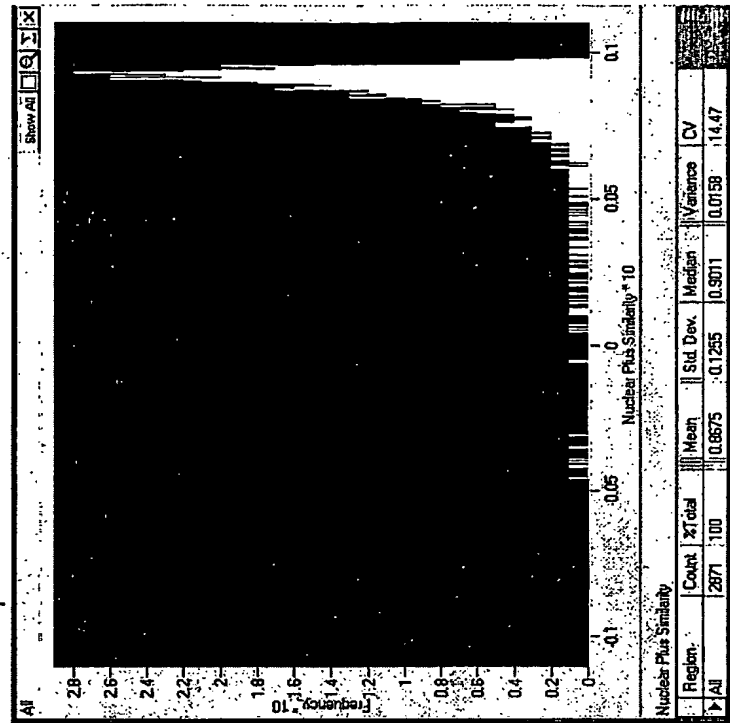
Compartmental Corelation Feature: A-549 Cells
Untreated



Median Compartmental Corelation Feature:
-2815 +/- 0.2806

Difference of 1.1826

IL-1 β /TNF- α -Treated



0.9011 +/- 0.1255

Fig. 13